Gram Stain Kit

Intended use
Ready to use combi kit each of Gram Crystal Violet (A), Gram Iodine (B), Gram Decolorizer (C) and Gram Safranin
(D) to differentiate between Gram-positive and Gram-negative bacteria.

Summary
Various mechanisms have been proposed to explain the Gram character. The characteristic color of Gram-positive bacteria results from the ability of a cell to resist the loss of the primary stain during the decolorization step, as compared to the gram-negative cells which do not possess this ability. Most researchers who have studied the mechanism of Gram stain reaction have concluded that the mechanism is based on the distinctive chemistry and physical structure of the cell walls of the organisms.

Principle
Gram staining is based on the ability of bacterial cell wall to retain the crystal violet dye during solvent treatment. The cell walls for Gram-positive microorganisms have a higher peptidoglycan and lower lipid content than Gram- negative bacteria. Bacteria cell walls are stained by the crystal violet. Iodine is subsequently added as a mordant to form the crystal violet-iodine (CV-I) complex so that the dye cannot be removed easily with solvent treatment. This step is commonly referred to as fixing the dye. Therefore, these cells remain purple violet and do not take up the counter stain. However, subsequent treatment with a decolorizer, which is a mixed solvent of ethanol and acetone, dissolves the lipid layer from the Gram-negative cells. The removal of the lipid layer enhances the leaching of the primary stain from the cells into the surrounding solvent. In contrast, the solvent dehydrates the thicker Gram-positive cell walls, closing the pores as the cell wall shrinks during dehydration. As a result, the diffusion of the violet-iodine complex is blocked, and the bacteria remain stained. The length of the decolorization is critical in differentiating the Gram-positive bacteria from the Gram-negative bacteria. Finally, a counterstain of safranin is applied to the smear to give decolorized Gram-negative bacteria a pink color.

Reagent / Contents
The Gram Stain Kit comprises of
Gram Crystal Violet Solution (Reagent A) Gram
Iodine Solution (Reagent B)
Gram Decolorizer (Reagent C) Gram Safranin
Solution (Reagent D)

Appearance
Gram Crystal Violet:	Dark violet coloured solution
Gram Iodine:	Dark amber coloured solution
Gram Decolourizer Solution:	Colourless clear solution
Gram Safranin 0.5 %:	Red coloured solution

Accessories:
Cap with dispenser-3 Nos.
Dropper- 1 No.

Storage and Stability
Store at 15°C- 25°C away from bright light. Stability of the Gram Stain Kit is as per expiry date mentioned on label.

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Materials required but not provided

Clean grease-free glass slide, loops, staining rack, Bunsen burner, blotting paper, immersion oil , microscope.

Type of Specimen

Bacterial smears of isolated colonies of Clinical samples - Blood, urine, CSF, body fluid specimens, biopsy specimens, positive culture specimens of pus, wounds, lesions, sputum etc.; food & dairy samples; Water samples.

Procedure

1. Prepare a thin smear on clear, dry glass slide.
2. Allow it to air dry and fix by gentle heat.
3. Flood with Gram's Crystal Violet (Reagent A) for 1 minute. (Over staining results in improper decolorization of known gram-negative organisms, use less crystal violet).
4. Wash with tap water.
5. Flood the smear with Gram's Iodine (Reagent B). Allow it to remain for 1 minute.
6. Decolorize with Gram's Decolorizer (Reagent C) until the blue dye no longer flows from the smear.
7. Wash with tap water.
8. Counter stain with 0.5% w/v Safranin (Reagent D) for 30 seconds and rinse off with water.
9. Wash with tap water.
10. Allow the slide to air dry or blot dry between sheets of clean bibulous paper and examine under oil immersion objective lens.

Interpretation of Results

Gram-positive organisms are stained bluish purple. Gram-negative organisms are stained pinkish red.

Precautions/Limitations

1. The Gram stain kit is an *in vitro* diagnostic kit for laboratory and professional use only. Not for medicinal use.
2. Reagents must be added in the order and the amounts specified or a weak-positive or false-negative reaction may occur.
3. At times, the organism may give contradictory results because of mutation or media used for isolation, cultivation and maintenance. Results are prominent when fresh and enriched culture is used.
4. Clinical samples and microbial cultures should be considered as pathogenic biohazard and handled accordingly. Good laboratory practices and hazard precautions must be observed at all times.
5. The test is an aid to identification and is not a confirmatory test. Complete identification should include determination of Gram reaction, morphology, and other biochemical and serological tests.
6. Do not use damaged or leaking kits. Avoid contact of reagents with skin and eyes.

Warranty

This product is designed to perform as described on the label and pack insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

References

1. Burke V 1992, Notes on the gram stain with description of a new method, Journal of Bacteriology, 7:159-182
2. Kopeloff N, Beerman P 1992, Modified gram stains, Journal of infectious diseases, 31;480-482
3. Data on file: UltraCare Diagnostics .

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